Colorectal Cancer and Folate Status: A Nested Case-Control Study among Male Smokers[1]

Simone A. Glynn, Demetrius Albanes,[2] Pirjo Pietinen, Charles C. Brown, Matti Rautalahti, Joseph A. Tangrea, Elaine W. Gunter, Michael J. Barrett, Jarmo Virtamo, and Philip R. Taylor

Division of Cancer Prevention and Control, National Cancer Institute, Bethesda, Maryland 20892 [S. A. G., D. A., C. C. B., J. A. T., P. R. T.]; National Public Health Institute, Helsinki SF-00300, Finland [P. P., M. R., J. V.]; Centers for Disease Control and Prevention, Atlanta, Georgia [E. W. G.]; and Information Management Services, Inc., Silver Spring, Maryland, 20910 [M. J. B.]

Abstract

Evidence is accumulating that folate, a B vitamin found in green leafy vegetables, may affect the development of neoplasia. We examined the relationship between folate status and colorectal cancer in a case-control study nested within the Alpha-Tocopherol Beta-Carotene Study cohort of male smokers 50–69 years old. Serum folate was measured in 144 incident cases (91 colon, 53 rectum) and 276 controls matched to cases on baseline age, clinic, and time of blood collection. Baseline dietary folate was available from a food-use questionnaire for 386 of these men (92%). Conditional logistic regression modeling was used. No statistically significant association was observed between serum folate and colon or rectal cancer. Although a 2-fold increase in rectal cancer risk was suggested for men with serum folate >2.9 ng/ml and those in the highest quartile of energy-adjusted folate intake, there was no evidence of a monotonic dose-response, and all confidence intervals included unity. For dietary folate and colon cancer, odds ratios of 0.40 [95% confidence interval (CI), 0.16–0.96], 0.34 (95% CI, 0.13–0.88), and 0.51 (95% CI, 0.20–1.31) were obtained for the second through fourth quartiles of energy-adjusted folate intake, respectively, compared to the first (*P* for trend = 0.15). Furthermore, men with a high-alcohol, low-folate, low-protein diet were at higher risk for colon cancer than men who consumed a low-alcohol, high-folate, high-protein diet (OR, 4.79; 95% CI, 1.36–16.93). This study suggests a possible association between low folate intake and increased risk of colon cancer (but not rectal cancer) and highlights the need for further studies that measure dietary folate and methionine, along with biochemical measures of folate (*i.e.*, erythrocyte and serum), homocysteine, and vitamin B_{12}.

Introduction

In Finland and much of the West, colorectal cancer is the third most common malignancy among men after lung and prostate cancer. Between 1988 and 1992, annual age-adjusted incidence rates among Finnish men were 13.0 and 10.5 per 100,000 for colon and rectal cancer, respectively (1). The considerable variation in colorectal cancer rates observed worldwide may be due to differences in dietary habits (2, 3). Although most studies have shown an inverse relationship between large bowel cancer and fruit and vegetable consumption, it remains unclear whether this protective association is secondary to specific constituents such as fiber, β-carotene, vitamin C, folate, or other micronutrients, or due to some combination of these factors (3–5).

Evidence is accumulating that folate, a water-soluble vitamin, may affect the development of neoplasia (6). Its role in colorectal carcinogenesis has been studied to a limited extent, primarily with respect to folate intake (*i.e.*, diet with or without supplements: Refs. 7–15). Two studies demonstrate an inverse association between folate and colon adenomas (8, 9), and of seven published reports concerning colorectal cancer (7, 10–15), three (7, 10, 12) show a protective role for folate. One study (14) showed increased colon cancer risk for subjects having low folate, low methionine, and high alcohol intakes, possibly reflecting the combined effect of these dietary factors on AdoMet,[3] a major methyl-group donor involved in DNA methylation and possibly colon carcinogenesis. The current debate concerning fortification of foods with folic acid (16–18) in response to evidence that folate prevents neural tube defects (19, 20) heightens the need for timely investigation of the potential impact of folate status on other major illnesses, including vascular diseases (21, 22) and cancer, so that public health repercussions associated with folic acid fortification are more thoroughly understood (23, 24).

We examined the relationship between folate status and colorectal cancer through a nested case-control study in a cohort of male smokers in Finland. The effect of a low-folate, low-protein (highly correlated with methionine), high-alcohol dietary pattern on colorectal cancer was also specifically investigated. Both serum folate and total folate intake (diet and supplements) were used as measures of folate status.

Materials and Methods

Study Population. We conducted a case-control investigation nested within the ATBC Cancer Prevention Study (25). This

Received 1/4/96; revised 4/8/96; accepted 4/9/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by National Cancer Institute Contract NO1-CN-45165.

[2] To whom requests for reprints should be addressed, at Cancer Prevention Studies Branch, Division of Cancer Prevention and Control, National Cancer Institute, Executive Plaza North, Suite 211, 9000 Rockville Pike, Bethesda, MD 20892-7326.

[3] The abbreviations used are: AdoMet, S-adenosylmethionine; ATBC, Alpha-Tocopherol Beta-Carotene; QC, quality control; OR, odds ratio; CI, confidence interval; RR, relative risk.

trial evaluated the effect of 50 mg/day α-tocopherol and 20 mg/day β-carotene on the occurrence of lung and other cancers using a 2 × 2 factorial design among 29,133 men between the ages of 50 and 69 who smoked at least 5 cigarettes/day and lived in southwestern Finland (25, 26). Men with malignancies (except nonmelanoma skin cancer and *in situ* carcinoma), severe angina with exertion, chronic renal insufficiency, alcoholism, cirrhosis, and those taking anticoagulants or supplementation with β-carotene or vitamin A or vitamin E in excess of defined amounts were excluded. Recruitment occurred between 1985 and 1988, and participants were followed up for 5–8 years. Before randomization, we documented baseline participant characteristics such as height, weight, medical history, and dietary habits during the past 12 months and collected serum samples storing them at −70°C (25). This clinical trial was approved by the institutional review boards of the National Cancer Institute and the National Public Health Institute of Finland.

Using the Finnish Cancer Registry (27), we identified all cases of incident colon (ICD-153) and rectal (ICD-154) cancer occurring from January 1985 through November 1993. We excluded 10 cases on the basis of histology (squamous cancers, carcinoid tumors, and *in situ* adenocarcinoma). For five cases having more than one malignant colorectal tumor, we retained the histology and diagnosis date pertaining to the earliest cancer diagnosed. Of the remaining 147 cases, 145 were adenocarcinomas, 1 was an anaplastic carcinoma, and 1 was unclassified. Using the incidence-density method (28–30), we matched control subjects from the ATBC Study cohort to case subjects in a 2:1 ratio on the basis of age (± 1 year), study center, and closest time of blood collection at baseline (± 1 month). Unavailable serum (empty or broken vials or insufficient volume) resulted in the exclusion of 3 case and 14 control subjects. Hence, the serum folate analyses used 276 control subjects (including 1 control used for 2 cases) matched to 144 case subjects (91 colon cancers and 53 rectal cancers). For all dietary analyses, we used the subset of men who had completed the dietary questionnaire (see below): 249 control subjects (including 1 duplicate) and 136 subjects with colorectal cancer (86 colon, 50 rectum).

Folate Status. Folate status was evaluated measuring serum folate and total folate (dietary and supplements) intake. Fasting blood samples were taken at the first baseline visit before intervention. Hemolyzed samples were discarded, and samples were kept in the dark and stored at −70°C. Because of possible degradation with time, even under these storage conditions, control subjects were matched to case subjects for time of blood drawing; this match also controlled for seasonal variation. All samples experienced an inadvertent quick thaw/freeze cycle during their shipment from Finland to the analytic laboratory at the Centers for Disease Control and Prevention. Serum folate was measured using the Bio-Rad laboratories Quantaphase II Folate radioassay kit with a reported detection limit of 0.1 ng/ml and average coefficient of variation of <6% across the range 1–20 ng/ml (31). In addition to the routine QC samples used in the laboratory at CDC, blind QCs were introduced in a ratio of ~1:10 in each batch to further evaluate reproducibility. Coefficients of variation were found to range from 4.6 to 8.8%.

Dietary folate was measured using information collected from a detailed food use questionnaire linked to the food composition data base of the National Public Health Institute in Finland. This questionnaire included a color picture booklet and asked about the usual frequency of consumption (over the previous 12 months) and portion sizes for 276 common foods and mixed dishes. When evaluated for accuracy, Pearson correlation coefficients ranged from 0.54 (vitamin A) to 0.9 (alcohol) for reproducibility and from 0.4 (selenium) to 0.8 (alcohol) for validity compared with 24 days of food records (32). After updating our food composition data base to include folate, analyses were rerun, and correlation coefficients of 0.70 on average for reproducibility and 0.54 for validity were obtained for dietary folate. Information concerning the use of folate supplements was obtained at baseline through a question about vitamin supplements, including type of supplement and frequency of intake. Folate supplements in the form of multivitamins (100 or 200 μg/day) were taken by only 5% (7 of 129) of case subjects and by 8% (20 of 249) of control subjects. We calculated total folate intake by combining dietary and supplemental intake.

Other Factors. Other variables that might confound or modify the association between folate and colorectal cancer were measured. From the food use questionnaire and food composition data base, we estimated intake of total energy, fat, fiber, protein, starch, vitamin A, vitamin C, vitamin E, calcium, iron, and alcohol. Also derived from a baseline questionnaire were education, degree of urbanization, physical activity, number of total cigarettes smoked, and smoking duration. Body mass index (kg/m^2) was calculated from baseline weight and height. Serum α-tocopherol, β-carotene, and cholesterol concentrations were available from baseline measurements. Treatment group assignment was also considered as a possible confounder or effect modifier.

Statistical Analysis. ORs were calculated using conditional logistic regression. To adjust all dietary variables for total energy, we used the residual method described by Willett and Stampfer (33) for the main models using transformations (logarithmic or square root) to preserve the linear model assumptions. Multivariable models were developed separately for each measure of folate (folate intake and serum levels). All dietary variables were introduced in the model as indicator variables representing quartile categories on the basis of the distribution of their residuals among all control subjects. Nondietary variables were introduced either as continuous variables or as indicator variables representing defined groups or quartile categories on the basis of the distribution among control subjects. Using a forward selection method, we kept in the final model variables that led to a significant change in likelihood ratios ($P \leq 0.05$) in either the colon or the rectal cancer analyses. The potential effect modification of the association between folate and colorectal cancer by other study factors was tested by including that factor and its interaction term(s) in the model or by stratification. Exposure scores to evaluate trend were based on the median values of the first to fourth quartiles under consideration. All analyses were conducted using SAS software (34, 35).

Results

Subjects with colorectal cancer and matched control subjects had similar distributions of age at randomization, physical activity during leisure time, and degree of urbanization; one-half were retired, and most had an elementary school education (Table 1). Control subjects were somewhat more physically active at work than were case subjects. At the beginning of the study, both case and control subjects were smoking an average of 20 cigarettes/day and had smoked for 38 years. Table 2 shows that dietary intake was similar for both case and control subjects, with modest differences noted for total energy and most nutrients, and somewhat higher alcohol consumption in cases. Compared to the usual United States dietary intake, these

Table 1 Distribution for covariables of interest (%)

Covariables		All control subjects (n = 276)	Colorectal cancer case subjects (n = 144)
Age at randomization (yr)	<55	21	22
	55–59	29	31
	60–64	28	25
	>65	22	22
Physical activity during work	Retired	54	51.5
	Sitting	9	17.5
	Light exercise	16	17
	Moderate exercise	13	8
	Heavy exercise	8	6
Physical activity during leisure	Reading	44	46
	Walking	50	46
	Exercise	6	8
Degree of urbanization	Countryside	19.5	18
	Village	12	13
	Small town	20	18
	Large town	48.5	51
Treatment group	Placebo	21	26
	α-Tocopherol	27	21
	β-Carotene and α-Tocopherol	21	26
	β-Carotene	31	27
Education	Less than elementary	6	4
	Elementary school	76	73
	Junior high school	10	15
	Senior high school	8	8

Table 2 Daily dietary intake[a]

Covariables	Control subjects, n = 249	Colorectal cancer case subjects, n = 136
Total energy, kcal	2620 (2144–3255)	2713 (2301–3247)
Fat, g	115 (93–145)	120 (97–146)
Fiber, g	24 (18–32)	25 (19–32)
Protein, g	97 (80–120)	101 (86–115)
Starch, g	134 (112–177)	148 (116–173)
Vitamin E, mg	11.0 (8.2–15.2)	10.6 (8.5–15.4)
Vitamin A, μg	1884 (1178–2642)	1934 (1116–3320)
Vitamin C, mg	91.6 (65.3–124.8)	97.3 (71.5–133.7)
Vitamin D, μg	5.00 (3.30–7.10)	4.94 (3.52–7.04)
Calcium, mg	1350 (985–1702)	1295 (986–1623)
Iron, mg	17.4 (14.2–21.6)	18.4 (14.4–22.2)
Alcohol, g	10.3 (3.1–22.9)	13.4 (3.2–28.1)

[a] Median (interquartile range).

diets were characterized by high intake of fiber (median, 24 g/day), fat (40% of kilocalories), calcium (median, 1.35 gm/day), and vitamin D (median, 5 μg/day). Although not shown, body mass index and serum α-tocopherol were similar in both groups at baseline; control subjects seemed to have slightly higher serum β-carotene levels: 201 *versus* 185 μg/liter for case subjects.

Serum folate concentration and total folate intake by case status are presented in Table 3. Compatible with other studies that have evaluated folate levels among smokers (36–38), serum folate concentrations were in the low-normal range [normal reference range, 1.8 ng/ml (5th percentile)–11.4 ng/ml (95th percentile); Ref. 39], and narrowly distributed. Median serum concentrations of case and control subjects were similar, albeit slightly higher among cases, and nearly identical among those for whom dietary data are available. [Mean concentrations (SD) corresponding to colorectal cancer cases and to control subjects were 4.41 (1.84) ng/ml and 4.28 (2.03) ng/ml, respectively.] Serum folate was not significantly correlated with any of the dietary study factors we evaluated, with the exception of energy-adjusted total folate intake [Spearman correlation coefficient (r) = 0.22; $P < 0.001$]. Serum folate was inversely correlated with years of smoking ($r = -0.12$; $P = 0.01$).

Total folate intake, unadjusted for energy intake, did not differ between case and control subjects with the exception that intake appeared higher among rectal cancer cases (348 g/day) compared to their matched control subjects (292 g/day). [Mean total folate intakes (SD) for case subjects with colorectal cancer and control subjects were 339 (±97) μg/day and 340 (±105) μg/day, respectively.] Excluding supplemental folate gave similar findings: median dietary intake of 325 μg/day for colorectal cases and 313 μg/day for control subjects. Compatible with the fact that folate is found in a variety of foods, total folate intake was significantly correlated ($P = 0.0001$) with total energy intake ($r = 0.78$) and with energy-adjusted intake of dietary fiber ($r = 0.46$), protein ($r = 0.29$), starch ($r = 0.35$), vitamin E ($r = 0.34$), vitamin A ($r = 0.23$), iron ($r = 0.45$), and alcohol ($r = -0.21$). Total folate intake was inversely correlated with smoking duration ($r = -0.13$; $P = 0.009$) and number of cigarettes/day ($r = -0.10$; $P = 0.05$). The strong correlation with energy intake necessitated the use of energy-adjusted folate intake in subsequent analyses of disease risk.

Table 4 shows the ORs for colon and rectal cancers by quartile of serum folate. The adjusted model contained total energy intake and energy-adjusted intake of vitamin A and starch. All analyses also inherently controlled for age, study center, and time of blood collection as a result of the matched design. Serum folate concentration was not significantly associated with either colon or rectal cancer. Although there was no

Table 3 Distribution of serum folate (ng/ml) and total folate intake (dietary and supplement in μg/day) by case status

	Control subjects, all	Colorectal cancers	Controls, colon cancer	Cases, colon cancer	Controls, rectal cancer	Cases, rectal cancer
Serum folate, all subjects						
n	276	144	175	91	101	53
Median	3.80	4.00	3.90	4.00	3.60	3.90
(Interquartile range)	(2.90–5.20)	(3.15–5.30)	(3.10–5.50)	(3.20–5.40)	(2.70–4.60)	(3.10–5.10)
Serum folate, dietary subgroup						
n	249	136	159	86	90	50
Median	3.80	4.00	3.90	4.00	3.70	3.90
(Interquartile range)	(2.90–5.30)	(3.20–5.25)	(3.10–5.60)	(3.20–5.30)	(2.70–4.90)	(3.20–5.10)
Total folate intake						
n	249	136	159	86	90	50
Median	320	327	330	322	292	348
(Interquartile range)	(263–403)	(267–392)	(276–408)	(267–379)	(254–392)	(261–401)

Table 4 ORs (95% CIs) by quartile of serum folate (ng/ml) for colon and rectal cancer

Quartiles[a]	Q1	Q2	Q3	Q4	Test for trend, *P* value
Colon					
All subjects[b]	1.00	0.67 (0.30–1.50)	1.26 (0.57–2.78)	0.92 (0.42–2.00)	0.87
No. case subjects	19	15	31	26	
Dietary subset[c]	1.00	0.61 (0.25–1.45)	1.25 (0.50–3.17)	0.96 (0.40–2.30)	0.83
No. case subjects	18	18	28	22	
Rectum					
All subjects[b]	1.00	1.88 (0.74–4.81)	1.78 (0.70–4.54)	2.04 (0.81–5.15)	0.15
No. case subjects	12	11	17	13	
Dietary subset[c]	1.00	2.21 (0.65–7.48)	2.43 (0.72–8.19)	2.94 (0.84–10.33)	0.10
No. case subjects	10	14	14	12	

[a] Quartiles of serum folate (ng/ml) defined as: ≤2.9, >2.9–≤3.8, >3.8–≤5.2, and >5.2.
[b] The model contains serum folate alone. Because of the nature of the match, ORs are already corrected for age, clinic, and time of blood collection.
[c] The model includes serum folate, total energy intake, and energy-adjusted intakes of vitamin A and starch (residuals).

Table 5 ORs[a] (95% CIs) for quartile of energy-adjusted total folate intake (dietary and supplements) for colon and rectal cancer

Quartile[a]		Q1	Q2	Q3	Q4	Trend test *P* value
Colon	Energy-adjusted[b]	1.00	0.47 (0.21–1.03)	0.47 (0.22–1.01)	0.52 (0.24–1.12)	0.07
	Fully-adjusted[c]	1.00	0.40 (0.16–0.96)	0.34 (0.13–0.88)	0.51 (0.20–1.31)	0.15
	No. of case subjects	29	17	19	21	
Rectum	Energy-adjusted[b]	1.00	0.53 (0.16–1.76)	1.17 (0.46–2.95)	1.13 (0.37–3.41)	0.66
	Fully-adjusted[c]	1.00	0.50 (0.11–2.32)	1.78 (0.42–7.59)	2.12 (0.43–10.54)	0.26
	No. of case subjects	14	7	17	12	

[a] For a fixed level of energy, men in the second, third and fourth quartiles of energy-adjusted total folate intake consumed 15%, 26% and 45% more folate, respectively, than men in the first quartile. Derived median values for each quartile were Q1 = 268 μg/d, Q2 = 308 μg/d, Q3 = 337 μg/d and Q4 = 388 μg/d.
[b] Model includes total energy intake and energy-adjusted intakes of total folate (residuals).
[c] Model includes physical activity during leisure, total energy intake, and energy-adjusted intakes of total folate, vitamin A, and starch (residuals).

dose response and trends were not significant, we observed an overall increase in OR for the second to fourth quartiles of serum folate for rectal cancer. Results suggested that subjects with serum folate >2.9 ng/ml had a 2-fold increase in risk of rectal cancer compared to subjects whose serum folate was ≤2.9 ng/ml. All CIs were wide and included 1.0, however, reflecting the small number of rectal cases. In an attempt to minimize the potential effect of preclinical disease on serum folate, we also conducted analyses that excluded the first 2 years of follow-up. ORs for the second through fourth quartiles were similar, and all 95% CIs included 1.0: for colon cancer, unadjusted ORs were 0.45, 1.23, and 0.73, and for rectal cancer, unadjusted ORs were 2.24, 1.74, and 2.43, respectively. Separate analyses that evaluated the association between serum folate and either proximal or distal colon cancer did not reveal any differential pattern between these two subtypes.

Table 5 presents ORs for colon and rectal cancer by quartile of energy-adjusted total folate intake and multivariate-adjusted folate calculated from models that included physical activity during leisure time, energy intake, and starch and vitamin A intake (each energy-adjusted). Men in the second through fourth quartile of energy-adjusted folate intake seemed to be at lower risk for colon cancer compared with those in the lowest quartile. The trend test was of borderline significance ($P = 0.07$), and full adjustment resulted in a similar threshold-like association. For a fixed level of energy intake, men who

Table 6 ORs (95% CIs) for colorectal, colon, and rectal cancer by alcohol-protein-folate status

			Low alcohol, high folate, high protein[a]	Intermediate	High alcohol, low folate, low protein
Total Folate Intake	Colon	Energy-adjusted[b]	1.00	1.21 (0.57–2.58)	3.74 (1.28–10.91)
		Fully-adjusted[c]	1.00	1.02 (0.44–2.37)	4.79 (1.36–16.93)
		No. of case subjects	8	54	24
	Rectum	Energy-adjusted[b]	1.00	0.90 (0.34–2.38)	0.52 (0.12–2.15)
		Fully-adjusted[c]	1.00	0.53 (0.15–1.89)	0.49 (0.08–2.87)
		No. of case subjects	12	25	13
Serum Folate	Colon	Energy-adjusted[b]	1.00	0.64 (0.29–1.39)	1.27 (0.38–4.22)
		Fully-adjusted[c]	1.00	0.53 (0.22 –1.24)	1.28 (0.34 –4.88)
		No. of case subjects	9	58	19
	Rectum	Energy-adjusted[b]	1.00	0.40 (0.13–1.24)	0.33 (0.06–1.82)
		Fully-adjusted[c]	1.00	0.30 (0.07 –1.21)	0.46 (0.07 –3.29)
		No. of case subjects	7	34	9

[a] Definition of high versus low was based on median values of protein (97 g/day), alcohol (10 g/day), and either total folate (320 μg/day) or serum folate (3.8 ng/ml) based on control distribution.
[b] Energy-adjusted model includes total energy intake.
[c] Fully adjusted model includes intake of total energy, vitamin A, and starch for serum folate with the addition of physical exercise (leisure) for total folate intake.

consumed 15% more folate (*i.e.*, quartiles 2, 3, and 4 compared to quartile 1) had a two-thirds to one-half reduction in colon cancer risk (Table 5). Preliminary analysis using a multivariate (as per the fully adjusted models in Table 5) proportional hazards model for colon cancer and the recently available total folate intake of the entire cohort gave somewhat attenuated RRs (CIs) for the second to fourth quartiles compared to the first: 0.79 (0.43–1.47), 1.08 (0.60–1.94), and 0.72 (0.38–1.36). Furthermore, subsite analysis of colon cancer revealed a similar threshold effect for distal tumors [second- to fourth-quartile energy-adjusted ORs: 0.25 (0.07–0.86), 0.23 (0.01–0.73), and 0.65 (0.21–2.05)] with a more attenuated effect for proximal colon cancer [second- to fourth-quartile energy-adjusted ORs: 1.02 (0.33–3.12), 0.92 (0.27–3.08), 0.38 (0.12–1.25)]. The latter analyses are based on relatively few cases, however.

No similar pattern was observed for rectal cancer. In fact, adjusted ORs suggested a 2-fold increase risk of rectal cancer among men in the third to fourth quartiles of energy-adjusted intake compared to men in the first quartile. These ORs were not, however, significantly different from 1. ORs calculated from a model that excluded the first 2 years of follow-up were similar to those presented in Table 5, and analyses that evaluated dietary folate alone (*i.e.*, without supplements) gave similar results (data not shown).

We evaluated whether the association between folate (serum or diet) and colorectal cancer was modified by any of the variables included in the final models or by other selected factors (*i.e.*, follow-up time, trial intervention group, total number of cigarettes/day, smoking duration, and alcohol and protein intake). None of the factors studied demonstrated an interaction with folate status and colon or rectal cancer. Because prior evidence suggested some benefit in colon cancer for a low-alcohol, high-methionine, high-folate diet (14), we further analyzed possible interactions between folate, alcohol, and protein intakes (the latter being highly correlated with the amino acid methionine). Risk of large bowel cancer among men consuming large amounts of alcohol and low amounts of folate and protein (as a surrogate measure of methionine) was compared to that of men with low alcohol intake and high intake of folate and protein (low/high defined by median intake for all control subjects). Similar analyses were conducted substituting serum folate for dietary folate.

As can be seen in Table 6, a pattern of high-alcohol, low-folate, and low-protein intake was associated with increased colon cancer risk when compared with a low-alcohol, high-folate, high-protein diet (OR, 4.79; 95% CI, 1.36–16.93). [We also found that the high-alcohol, low-folate, low-protein diet pattern was associated with increased risk of colon cancer compared to a low-alcohol, high-folate, high-protein diet for the entire cohort in the preliminary age- and energy-adjusted multivariate proportional hazards analysis (RR, 2.66; CI, 1.14–6.19)]. In contrast, these dietary patterns were not associated with rectal cancer. Analysis of serum folate suggested that men characterized by a high-alcohol, low-protein diet and serum folate concentrations <3.8 ng/ml had a 28% elevation in risk for colon cancer but were at approximately half the risk for rectal cancer compared with men in the opposite group (low-alcohol, high-protein diet and serum folate ≥3.8 ng/ml). None of these ORs differed significantly from 1, however.

Discussion

We did not observe a significant association between serum folate and colon cancer in male smokers. Our results suggest, however, that higher folate consumption relative to energy intake may be protective for colon cancer, because we observed a one-half to two-thirds reduction in risk in persons consuming at least 15% more folate than persons in the lowest quartile of energy-adjusted intake. Previous studies have reported an inverse association between colon cancer and folate intake. In a case-control study conducted in Majorca, Benito *et al.* (12) reported an OR of 0.62 and nonsignificant trend comparing the high and low quartiles of intake, whereas Meyer and White (11) found an inverse association between folate intake and colon cancer among women only (OR, 0.73, comparing fourth with first quartile) in a case-control study based in Washington state. The inverse association noted by Ferraroni *et al.* (Ref. 15; OR, 0.55; 95% CI, 0.41–0.75) disappeared after adjustment for intake of carotene, ascorbic acid, and vitamin E. With respect to rectal cancer, there was no significant association for either total folate intake or serum folate, although a positive association was suggested, with men having serum concentrations of >2.9 ng/ml and men consuming at least 26% more folate than men in the lowest quartile of folate intake (for a fixed level of energy) being at highest risk. These results are not, however, consistent with other studies that have found 50–70% risk reductions in rectal cancer for high compared with low folate intake (10, 12). Although the present findings can be explained by chance, considering the wide CIs observed and lack of significant trends, data from other studies will be needed to clarify the role of folate (if any) in rectal carcinogenesis.

Our study design facilitated the prediagnostic evaluation of two measures of folate status, dietary folate intake, and serum folate levels. Thus, dietary information was not subject to recall bias, and serum folate was less likely to have been affected by the presence of colorectal cancer. We further reduced the possibility of such bias by excluding the first 2 years of follow-up and observed similar findings. The use of a detailed and validated dietary instrument that included most of the common foods consumed in Finland permitted evaluation of several nutrients that could confound or modify the association observed between folate intake and colorectal cancer.

The narrow range in serum folate values obtained was somewhat surprising, considering the wide distribution of total folate intake. This confined range may in part be secondary to the effect of smoking, which has been associated with lower serum folate concentrations (36–38), or reflect possible degradation of folate during storage or the inadvertent thaw/freeze cycle. We had matched for storage time because of uncertainty concerning possible degradation in serum folate during the average of 6 years of storage at −70°C. That little, if any, degradation occurred during storage or transport is supported by the comparison of our sample mean (4.3 ng/ml) with serum folate values (RIA) available for 200 of these participants from baseline samples analyzed 3.5 years later, or on average 2.5 years before our samples were analyzed (mean of 4.8 ng/ml). Regardless of its cause, the relatively narrow range may have diminished our ability to quantify the relationship between colorectal cancer, and particularly, higher serum folate concentrations.

The disparity in our findings for serum and dietary folate, and more specifically, the lack of an inverse association between serum folate and colon cancer, may also be explained in part by the fact that dietary and serum folate measure different aspects of folate status. Serum folate is low not only when folate stores are depleted but also in early negative folate balance (40). If consumption or excretion of folate is higher than absorption for 2–3 weeks or longer, serum folate will decrease, although folate stores may still be intact. Serum folate may therefore not be as good a marker of body folate storage as our measure of dietary folate, which reflected the usual folate intake over the previous 12 months. Erythrocyte folate is a better indicator of folate storage (40–42), but measurement requires the use of specially prepared specimens (*i.e.*, blood stored with ascorbic acid) that were not available in our study.

Dietary folate as measured by the food use questionnaire linked to the food composition data base was the major component of total folate intake in our population. The range we observed for total folate intake (102–693 μg/day) is similar to that obtained in some studies that measured only dietary sources (10), but lower than that reported from a cohort study conducted in the United States that included supplement use (8). Thus, our assessment of the consequences of higher folate intake may have been somewhat reduced. Dietary folate measurement is subject to variability because of the inherent difficulties associated with the use of food frequency questionnaires, as well as inaccuracies in available folate food composition data (43). Although not precise, our measurement of dietary folate intake provides a reasonable estimate of long-term intake and is adequate for group (*e.g.*, quartile) comparisons that are less affected by imprecision.

Folate status depends not only on dietary intake but also on absorption and metabolism, as exemplified by the modulating effect of smoking on serum levels. The correlation we obtained between folate intake and serum measures ($r = 0.22$) was the same as that obtained by VanEenwyk *et al.* (44). Correlations between folate intake and erythrocyte values tend to be higher and ranged from 0.27 (44) to 0.56 (8). Furthermore, dietary folate could be a marker for the intake of closely related compounds found in folate-rich foods, and not itself responsible for the observed associations.

Several mechanisms by which low-folate status could promote carcinogenesis have been proposed (45). The role of folate in DNA methylation has been of particular interest because several studies have noted abnormal DNA methylation patterns in colorectal adenomas and adenocarcinomas (46–51). The transfer of a one-carbon group from 5-methyl-tetrahydrofolate permits transformation of homocysteine into methionine, a precursor of AdoMet. AdoMet is a major methyl-group donor, and interference with its production by, for example, inadequate folate or methionine levels could lead to abnormalities in DNA methylation. Indeed, rats fed lipotrope-deficient diets (*i.e.*, low in methionine, choline, folic acid, and vitamin B_{12}) have lower hepatic levels of AdoMet (52, 53) and higher DNA hypomethylation and levels of c-myc, c-fos, and c-Ha-ras mRNAs than do adequately supplemented control rats (53). Addressing the role of folate more specifically, Cravo *et al.* (51) showed that folic acid (10 mg/day for 6 months) could reduce the level of DNA hypomethylation present in the normal-appearing rectal mucosa of people with adenomas or adenocarcinomas.

More recently, Laird *et al.* (54) have questioned the role of DNA hypomethylation in carcinogenesis, proposing instead that cytosine-to-thymine mutations may be an important factor in tumorigenesis. These investigators demonstrated a direct relationship between DNA-methyltransferase activity and incidence of intestinal adenomas in mice carrying an APC tumor suppressor gene mutation and suggested increased deamination of cytosine by DNA methyltransferase-cytosine adducts resulting from decreased AdoMet levels as a possible mechanism (54). Reduction of AdoMet may therefore be associated with tumorigenesis not because of hypomethylation, but because of an increase in cytosine mutation rates. Other studies are needed to further our understanding of these and other possible mechanisms. For example, alcohol may be another compound associated with reduced AdoMet levels (55), although it is unclear whether it is directly responsible for the observed decrease in levels or whether the effect is secondary to the diminished folate levels (56–58) or loss of methionine (58) associated with alcohol consumption.

Giovannucci *et al.* (14) have suggested that people who consume high-alcohol, low-methionine, low-folate diets and have relatively low levels of AdoMet may be at higher risk for colon cancer than people who consume a diet low in alcohol but high in folate and methionine, a hypothesis supported by their data (RR, 2.34; 95% CI, 1.23–4.30). Freudenheim *et al.* (10) had also noted that men who consumed high-alcohol, low-folate diets had higher risks for rectal cancer (OR, 5.07; 95% CI, 2.17–11.86) than men who had low alcohol and high folate intake (10). Although we did not observe a similar risk pattern for rectal cancer, our findings support the hypothesis that diets associated with lower AdoMet levels may be detrimental for colon cancer.

Our results indicate that dietary folate, and in particular a low-alcohol, high-folate, high-protein dietary pattern, may have a protective role in colon cancer, consistent with some previous reports. A similar relation was not observed for rectal cancer; instead, a positive (although not statistically significant) association was suggested for serum and dietary folate for this site, a finding that warrants evaluation in other investigations. Additional studies that measure dietary folate, dietary methionine, erythrocyte and serum folate concentrations, and homocysteine and vitamin B_{12} levels, would also prove helpful in further evaluating the role of folate in colorectal carcinogenesis and in defining the relative importance of specific dietary patterns related to mechanisms.

Acknowledgments

We thank Della Twite at the Division of Environmental Health Laboratory Sciences, Center for Disease Control and Prevention for technical assistance in the folate assays and Dr. Lisa McShane at the National Cancer Institute for analysis of laboratory QC data.

References

1. Finnish Cancer Registry. Cancer Incidence in Finland 1992: Cancer Statistics of the National Research and Development Center for Welfare and Health. Cancer Society of Finland Publ. 54. Helsinki: Cancer Society of Finland, 1994.

2. Boyle, P., Zaridze, D. G., and Smans, M. Descriptive epidemiology of colorectal cancer. Int. J. Cancer, *36:* 9–18, 1985.

3. Potter, J. D., Slattery, M. L., Bostick, R. M., and Gapstur, S. M. Colon cancer: a review of the epidemiology. Epidemiol. Rev., *15:* 499–545, 1993.

4. Schatzkin, A., Schiffman, M., and Lanza, E. Research priorities in large bowel cancer prevention. Semin. Oncol., *17:* 425–437, 1990.

5. Block, G., Patterson, B., and Subar, A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr. Cancer, *18:* 1–29, 1992.

6. Glynn, S. A., and Albanes, D. Folate and cancer: a review of the literature. Nutr. Cancer, *22:* 101–119, 1994.

7. Lashner, B. A. Red blood cell folate is associated with the development of dysplasia and cancer in ulcerative colitis. J. Cancer Res. Clin. Oncol., *119:* 549–554, 1993.

8. Giovannucci, E., Stampfer, M. J., Colditz, G. A., Rimm, E. B., Trichopoulos, D., Rosner, B. A., Speizer, F. E., and Willett, W. C. Folate, methionine, and alcohol intake and risk of colorectal adenoma. J. Natl. Cancer Inst., *85:* 875–883, 1993.

9. Benito, E., Cabeza, E., Moreno, V., Obrador, A., and Bosch, F. X. Diet and colorectal adenomas: a case-control study in Majorca. Int. J. Cancer, *55:* 213–219, 1993.

10. Freudenheim, J. L., Graham, S., Marshall, J. R., Haughey, B. P., Cholewinski, S., and Wilkinson, G. Folate intake and carcinogenesis of the colon and rectum. Int. J. Epidemiol., *20:* 368–374, 1991.

11. Meyer, F., and White, E. Alcohol and nutrients in relation to colon cancer in middle-aged adults. Am. J. Epidemiol., *138:* 225–236, 1993.

12. Benito, E., Stiggelbout, A., Bosch, F. X., Obrador, A., Kaldor, J., Mulet, M., and Muñoz, N. Nutritional factors in colorectal cancer risk: a case-control study in Majorca. Int. J. Cancer, *49:* 161–167, 1991.

13. Lashner, B. A., Heidenreich, P. A., Su, G. L., Kane, S. V., and Hanauer, S. B. Effect of folate supplementation on the incidence of dysplasia and cancer in chronic ulcerative colitis. Gastroenterology, *97:* 255–259, 1989.

14. Giovannucci, E., Rimm, E. B., Ascherio, A., Stampfer, M. J., Colditz, G. A., and Willett, W. C. Alcohol, low-methionine-low-folate diets, and risk of colon cancer in men. J. Natl. Cancer Inst., *87:* 265–273, 1995.

15. Ferraroni, M., La Vecchia, C., D'Avanzo, B., Negri, E., Franceschi, S., and Decarli, A. Selected micronutrient intake and the risk of colorectal cancer. Br. J. Cancer, *70:* 1150–1155, 1994.

16. Burk, D. T., and Mirkes, P. E. Folic acid prevention of neural tube defects: public policy issues. Teratology, *49:* 239–241, 1994.

17. Wald, N. J., and Bower, C. Folic acid, pernicious anaemia, and prevention of neural tube defects. Lancet, *343:* 307, 1994.

18. Wald, N. Folic acid and the prevention of neural tube defects. Ann. NY Acad. Sci., *678:* 112–129, 1993.

19. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. Lancet, *338:* 131–137, 1991.

20. Centers for Disease Control. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR (Morb. Mortal. Wkly Rep.), *41:* 1–7, 1992.

21. Brattstrom, L. E., Israelsson, B., Jeppsson, J. O., and Hultberg, B. L. Folic acid: an innocuous means to reduce plasma homocysteine. Scand. J. Clin. Lab. Invest., *48:* 215–221, 1988.

22. Clarke, R., Daly, L., Robinson, K., Naughten, E., Cahalane, S., Fowler, B., and Graham, I. Hyperhomocysteinemia: an independent risk factor for vascular disease. N. Engl. J. Med., *324:* 1149–1155, 1991.

23. Zimmermann, M. B., and Shane, B. Supplemental folic acid. Am. J. Clin. Nutr., *58:* 127–128, 1993.

24. Chanarin, I. Adverse effects of increased dietary folate, relation to measures to reduce the incidence of neural tube defects. Clin. Invest. Med., *17:* 244–252, 1994.

25. The ATBC Cancer Prevention Study group. The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics, and compliance. Ann. Epidemiol., *4:* 1–10, 1994.

26. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The Effect of vitamin E and β-carotene on the incidence of lung cancer and other cancers in male smokers. N. Engl. J. Med., *330:* 1029–1035, 1994.

27. Kyllonen, L. E. J., Teppo, L., and Lehtonen, M. Completeness and accuracy of registration of colorectal cancer in Finland. Ann. Chir. Gynaecol., *76:* 185–190, 1987.

28. Lubin, J. H., and Gail, M. H. Biased selection of controls for case-control analyses of cohort studies. Biometrics, *40:* 63–75, 1984.

29. Robins, J. M., Gail, M. H., and Lubin, J. H. More on "biased selection of controls for case-control analyses of cohort studies." Biometrics, *42:* 293–299, 1986.

30. Pearce, N. Incidence density matching with a simple SAS computer program. Int. J. Epidemiol., *18:* 981–984, 1989.

31. Gunter, E. W., Lewis, B. G., and Koncikowski, S. M. Laboratory methods used for the Third National Health and Nutrition Examination Survey (NHANES III), 1988–1994. Hyattsville, MD: Centers for Disease Control and Prevention, United States Public Health Service, in press, 1996.

32. Pietinen, P., Hartman, A. M., Haapa, E., Rasanen, L., Haapakoski, J., Palmgren, J., Albanes, D., Virtamo, J., and Huttunen, J. K. Reproducibility and validity of dietary assessment instruments. Am. J. Epidemiol., *128:* 655–666, 1988.

33. Willett, W., and Stampfer, M. J. Total energy intake: implications for epidemiologic analyses. Am. J. Epidemiol., *124:* 17–27, 1986.

34. SAS Institute, Inc. SAS/STAT User's Guide, Version 6, Vols. 1 and 2, Ed. 4. Cary, NC: SAS Institute, Inc., 1989.

35. SAS Institute, Inc. SAS Technical Report P-217, SAS/STAT Software. The PHREG Procedure, Version 6, pp. 1–59. Cary, NC: SAS Institute, Inc., 1991.

36. Nakazawa, Y., Chiba, K., Imatoh, N., Kotorii, T., Sakamoto, T., and Ishizaki, T. Serum folic acid levels and antipyrine clearance rates in smokers and nonsmokers. Drug Alcohol Depend., *11:* 201–207, 1983.

37. Witter, F. R., Blake, D. A., Baumgardner, R., Mellits, E. D., and Niebyl, J. R. Folate, carotene and smoking. Am. J. Obstet. Gynecol., *144:* 857, 1982.

38. Ortega, R. M., Lopez-Sobaler, A. M., Gonzalez-Cross, M. M., and Redondo, R. M. Influence of smoking on folate intake and blood folate concentrations in a group of elderly Spanish men. J. Am. Coll. Nutr., *13:* 68–72, 1994.

39. Raiten, D. J., and Fisher, K. D. Assessment of folate methodology used in the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994). J. Nutr., *125:* 1371S–1398S, 1995.

40. Herbert, V. Development of human folate deficiency. *In:* M. F. Picciano, E. L. R. Stokstad, and J. F. Gregory III (eds.), Folic Acid Metabolism in Health and Disease, pp. 195–210. New York: Wiley-Liss, 1990.

41. Herbert, V. The 1986 Herman Award Lecture. Nutrition science as a continually unfolding story: the folate and vitamin B_{12} paradigm. Am. J. Clin. Nutr., *46:* 387–402, 1987.

42. Herbert, V. Making sense of laboratory tests of folate status: folate requirements to sustain normality. Am. J. Hematol., *26:* 199–207, 1987.

43. Gregory, J. F. Chemical and nutritional aspects of folate research: analytical procedures, methods of folate synthesis, stability, and bioavailability of dietary folates. Adv. Food Nutr. Res., *33:* 1–101, 1989.

44. VanEenwyk, J., Davis, F. G., and Colman, N. Folate, vitamin C, and cervical intraepithelial neoplasia. Cancer Epidemiol., Biomarkers & Prev., *1:* 119–124, 1992.

45. Eto, I., and Krumdieck, C. L. Role of vitamin B_{12} and folate deficiencies in carcinogenesis. Advances in Experimental Medicine and Biology, *206:* 313–330, 1986.

46. Hoffman, R. M. Altered methionine metabolism and transmethylation in cancer. Anticancer Res., *5:* 1–30, 1985.

47. Feinberg, A. P., and Vogelstein, B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature (Lond.), *301:* 89–92, 1983.

48. Feinberg, A. P., Gehrke, C. W., Kuo, K. C., and Ehrlich, M. Reduced genomic 5-methylcytosine content in human colonic neoplasia. Cancer Res., *48:* 1159–1161, 1988.

49. Sharrard, R. M., Royds, J. A., Rogers, S., and Shorthouse, A. J. Patterns of methylation of the C-*myc* gene in human colorectal cancer progression. Br. J. Cancer, *65:* 667–672, 1992.

50. El-Deiry, W. S., Nelkin, B. D., Celano, P., Chiu Yen, R., Falco, J. P., Hamilton, S. R., and Baylin, S. B. High expression of the DNA methyltransferase gene characterizes human neoplastic cells and progression stages of colon cancer. Proc. Natl. Acad. Sci. USA, *88:* 3470–3474, 1991.

51. Cravo, M., Fidalgo, P., Pereira, A. D., Gouveia-Oliveira, Chaves P., Selhub, J., Mason, J. B., Mira, F. C., and Leitao, C. N. DNA methylation as an intermediate biomarker in colorectal cancer: modulation by folic acid supplementation. Eur. J. Cancer Prev., *3:* 473–479, 1994.

52. Shivapurkar, N., and Poirier, L. A. Tissue levels of *S*-adenosylmethionine and *S*-adenosylhomocysteine in rats fed methyl-deficient amino acid-defined diets for one to five weeks. Carcinogenesis (Lond.), *4:* 1051–1057, 1983.

53. Wainfan, E., and Poirier, L. A. Methyl groups in carcinogenesis: effects on DNA methylation and gene expression. Cancer Res., *52* (Suppl.): 2071s–2077s, 1992.

54. Laird, P. W., Jackson-Grusby, L., Fazeli, A., Dickinson, S. L., Jung, W. E., Li, E., Weinberg, R. A., and Jaenisch, R. Suppression of intestinal neoplasia by DNA hypomethylation. Cell, *81:* 197–206, 1995.

55. Lieber, C. S., Casini, A., DeCarli, L. M., Kim, C. I., Lowe, N., Sasaki, R., and Leo, M. A. *S*-Adenosyl-L-methionine attenuates alcohol-induced liver injury in the baboon. Hepatology, *11:* 165–172, 1990.

56. Shaw, S., Jayatilleke, E., Herbert, V., and Colman, N. Cleavage of folates during ethanol metabolism. Biochem. J., *257:* 277–280, 1989.

57. Hillman, R., McGuffin, R., and Campbell, C. Alcohol interference with the folate enterohepatic cycle. Trans. Assoc. Am. Physicians, *90:* 145–156, 1977.

58. Weir, D. G., McGing, P., and Scott, J. M. Folate metabolism, the enterohepatic circulation and alcohol. Biochem. Pharmacol., *34:* 1–7, 1985.